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Published in:
Molecular Microbiology

DOI:
[10.1111/j.1365-2958.2011.07861.x](https://doi.org/10.1111/j.1365-2958.2011.07861.x)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2011

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Ajon, M., Froels, S., van Wolferen, M., Stoecker, K., Teichmann, D., Driessen, A. J. M., Grogan, D. W., Albers, S-V., Schleper, C., & Ajon, M. (2011). UV-inducible DNA exchange in hyperthermophilic archaea mediated by type IV pili. *Molecular Microbiology*, 82(4), 807-817. <https://doi.org/10.1111/j.1365-2958.2011.07861.x>

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UV-inducible DNA exchange in hyperthermophilic archaea mediated by type IV pili

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Summary

Archaea, like bacteria and eukaryotes, contain proteins involved in various mechanisms of DNA repair, highlighting the importance of these processes for all forms of life. Species of the order *Sulfolobales* of hyperthermophilic crenarchaeota are equipped with a strongly UV-inducible type IV pilus system that promotes cellular aggregation. Here we demonstrate by fluorescence *in situ* hybridization that cellular aggregates are formed based on a species-specific recognition process and that UV-induced cellular aggregation mediates chromosomal marker exchange with high frequency. Recombination rates exceeded those of uninduced cultures by up to three orders of magnitude. Knockout strains of *Sulfolobus acidocaldarius* incapable of pilus production could not self-

aggregate, but were partners in mating experiments with wild-type strains indicating that one cellular partner can mediate the DNA transfer. Since pilus knockout strains showed decreased survival upon UV treatment, we conclude that the UV-inducible DNA transfer process and subsequent homologous recombination represents an important mechanism to maintain chromosome integrity in *Sulfolobus*. It might also contribute substantially to the frequent chromosomal DNA exchange and horizontal gene transfer in these archaea in their natural habitat.

Introduction

The damaging effect of UV irradiation on the integrity of DNA is well known and the understanding of the repair mechanisms in organisms that are exposed to sunlight is of fundamental interest, in particular since the depletion of the Earth's ozone layer increases the deleterious effect of solar radiation (McKenzie *et al.*, 2007). Bacteria, Archaea and Eukaryotes all have large numbers of proteins that are involved in DNA repair pathways of different types, reflecting the importance of these mechanisms for all life forms (White, 2003). Some archaea have photolyases that directly repair DNA photoproducts, such as cyclobutyl pyrimidine dimers (CPD). All seem to contain nucleotide excision repair systems that allow repair of UV-induced DNA lesions and also lesion bypass polymerases are found widespread, which bypass unrepaired damage during replication (Boudsocq *et al.*, 2001). The information processing machineries of archaea are generally most closely related to eukaryotes and also the DNA repair systems are most similar to the eukaryotic counterparts or only occur in the eukaryotic/archaeal lineages, such as the XPF proteins of the nucleotide excision repair (White, 2003). The proteins RadA, Mre11, Rad50, and the archaea-specific nuclease NurA and helicase HerA are most probably involved in DNA end repair (Quaiser *et al.*, 2008; Delmas *et al.*, 2009).

In a recent genome-wide microarray study of the UV response, we and others have shown that the hyperthermophilic archaeon *Sulfolobus* does not express an inducible set of proteins known to be involved in DNA repair, which would be comparable to an SOS response in bacteria. Instead the most strongly transcriptionally

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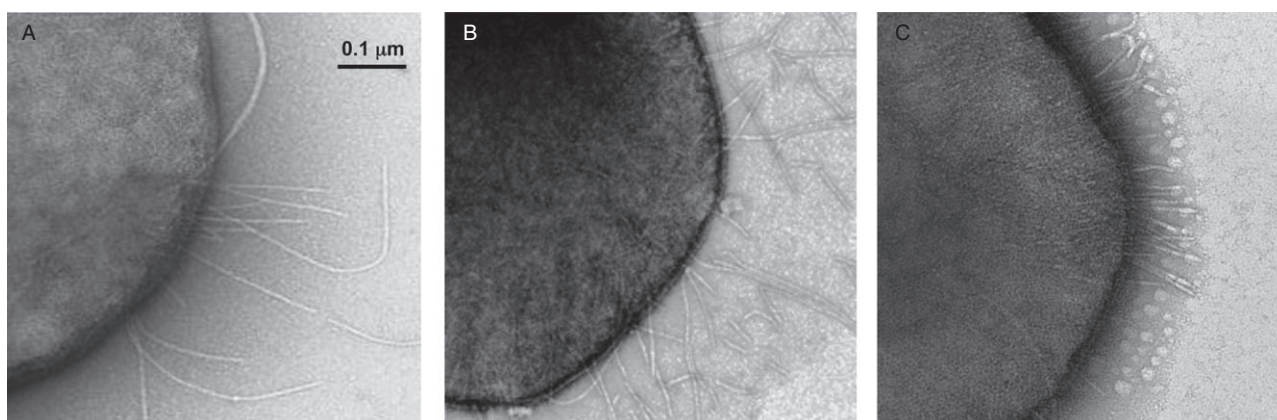


Fig. 1. UV-inducible pili on electron micrographs of *S. solfataricus* (A), *S. tokodaii* (B) and *S. acidocaldarius* (C). Pili exhibit similar diameters, but differences in length between the strains, with *S. acidocaldarius* producing the shortest but also most abundant pili.

induced genes were of unknown function or encoded functions that are not normally associated with a UV-specific response (Fröls *et al.*, 2007; Gotz *et al.*, 2007). Among the most strongly induced genes was the *ups* operon (UV-induced pili of *Sulfolobus*) encoding a type IV pili assembly system, which consists of a secretion ATPase UpsE, an integral transmembrane protein UpsF, two pilin-like proteins UpsA and B and a protein of unknown function UpsX (Fröls *et al.*, 2008). The *ups* operon was shown to assemble pili on the cell surface of *Sulfolobus solfataricus* which led to pili-mediated cell aggregation after UV stress (Fröls *et al.*, 2008). Type IV pili represent multifunctional filaments that are involved in bacterial twitching and gliding motility, surface and cell to cell adhesion, immune escape, formation of biofilm, secretion, phage and signal transduction and DNA uptake in various bacteria, as opposed to the type IV secretion systems that are involved in conjugation processes (Filloux, 2010). All sequenced *Sulfolobales* genomes possess the *ups* operon.

In addition we have demonstrated that DNA double-strand breaks occurred in the cells between 2 and 6 h after UV treatment, but not directly after the irradiation, indicating that they were a product of cellular repair or of replication processes rather than of direct damage (Fröls *et al.*, 2007). Similar observations of DNA double-strand breaks have been made decades ago in *Escherichia coli* (Bonura and Smith, 1975) and more recently also in human skin cells (Garinis *et al.*, 2005), indicating that their formation is a common but indirect and somewhat under-explored consequence of UV irradiation that could favour homologous recombination in the cells.

Here we demonstrate that the pili-dependent UV-induced cellular aggregation leads to efficient exchange of chromosomal markers among *Sulfolobus* cells of the same, but not of different species. The knockout of pili abolished DNA exchange and led to decreased survival

upon UV exposure, indicating that intra-species DNA exchange increases fitness of the cells. DNA repair based on homologous recombination might be active potentially at regions with DNA double-strand breaks formed after failed attempts to repair DNA by direct repair mechanisms.

Results

UV-induced pilus formation and cellular aggregation in *Sulfolobales*

In order to explore and compare the extent of pili formation in different species of *Sulfolobales*, cells of *Sulfolobus acidocaldarius*, *S. tokodaii* and *S. solfataricus* (Table 3) were exposed to UV-C light (dose 50 J m⁻²) and were inspected for pili formation by electron microscopy. Pili were found abundantly on all strains after UV exposure (Fig. 1), but not on untreated cells. The length of the *S. tokodaii* pili appeared with max 0.2 μm comparable to the *S. solfataricus* pili (length 0.2–0.3 μm) (Fröls *et al.*, 2008), whereas the UV-induced pili of *S. acidocaldarius* were only up to 0.1 μm in length, but more abundant. Pili were spread over the whole-cell surface sometimes as small bundles, but were not polarized at one side of the cell. The differences in pilus morphology of the three strains might be due to the differences in the amino acid sequence of the pilins (between 61% and 66% amino acid similarity, see Fig. S1) or due to different levels of their expression.

The three strains produced large amounts of cellular aggregates upon UV irradiation. These were particularly large in *S. acidocaldarius* with up to approximately 50 cells or more per aggregate (Fig. 2). One hour after UV irradiation with a dose of 50 J m⁻² already 30% of the cells were found in aggregates and later more than 90% (Fig. S2). These numbers are rather underestimates,

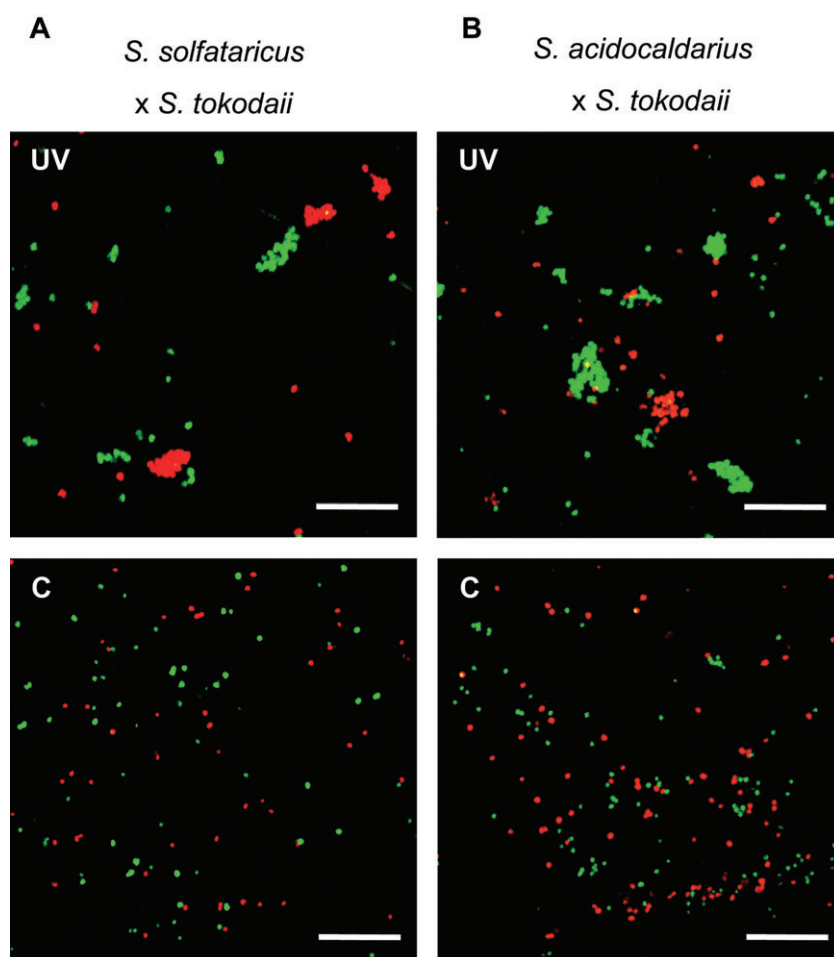


Fig. 2. Fluorescent *in situ* hybridization (FISH) with species-specific probes. UV-irradiated mixture of cells (UV) and control cultures (C) of *S. solfataricus* (green) with *S. tokodaii* (red) (A) and *S. acidocaldarius* (green) with *S. tokodaii* (red) (B). When cells were mixed, mostly homologous aggregates were formed, indicating a highly species-specific recognition mechanism (UV). Scale bar corresponds to 10 μm .

because the amount of cells in large aggregates was difficult to count.

The process of aggregation was time-dependent with a maximum of cellular aggregation observed from 3 to 6 h after UV treatment, when nearly all cells were found in aggregates. Although a basic level of around 10–20 cells in aggregates per 100 free cells were found in non-irradiated control cultures, those were considerably smaller than UV-induced aggregates (Fig. S2). Deaggregation started from 8 to 10 h after UV treatment and no aggregates were found any more 24 h after UV treatment. A similar trend had been observed with UV-induced aggregations in *S. solfataricus* (Fröls *et al.*, 2007). Aggregate formation depended on the UV dose, with the largest number of aggregates occurring between 25 and 75 J m^{-2} (Fig. S3). As demonstrated by a LiveDead stain with fluorescent dyes, at least 80% of the cells in aggregates of *S. acidocaldarius* exposed to a UV dose of 50 J m^{-2} were viable, i.e. had an intact plasma membrane (Fig. S4) indicating that the process of aggregation was not caused by accumulation of UV-destroyed and dead cells but reflected a specific response to UV stress as in *S. solfataricus* (Fröls *et al.*,

2008). Aggregation was also inducible by the DNA strand-break inducing agent bleomycin at non-lethal concentrations (Fig. S5). Aggregates appeared first 3 h after DNA-damaging treatment with 30 $\mu\text{g ml}^{-1}$ bleomycin, with a maximum number of aggregates after 6–8 h. Correspondingly, pili were observed in electron microscopy on the cells treated with bleomycin. The DSB-inducing agents bleomycin and mitomycin C had been observed to also induce cellular aggregation of *S. solfataricus* strains P1, PH1-M16 and PBL2025, but not of the knockout strain of the ATPase UpsE (Table 3) (Fröls *et al.*, 2008).

Species-specific aggregation

In order to explore the specificity of cell–cell recognition among UV-induced *Sulfolobus* cells, we have designed species-specific oligonucleotides targeting the small subunit ribosomal RNA in fluorescent *in situ* hybridizations (FISH). The probes had two to three mismatches each to the two other related species (Table S1).

Their specificity was confirmed by FISH analyses on pure cultures that were competitively challenged with the

Table 1. Recombination frequencies in *S. solfataricus* upon UV treatment with 75 J m⁻² UV (A, B) or 50 J m⁻² (C, D).

Experiment		Recombination mix cfu ml ⁻¹ (<i>lacS</i> ⁺ / <i>pyrEF</i> ⁻) × (<i>lacS</i> ⁺ / <i>pyrEF</i> ⁺)	Recombination frequencies ^a (<i>lacS</i> ⁺ / <i>pyrEF</i> ⁻)	Mutation frequencies ^b (<i>lacS</i> ⁺ / <i>pyrEF</i> ⁻)
A	75 J m ⁻²	3.80 × 10 ⁷	1.11 × 10 ⁻²	1.90 × 10 ⁻⁵
	Control	1.87 × 10 ⁸	— ^c	1.59 × 10 ⁻⁵
B	75 J m ⁻²	2.77 × 10 ⁷	4.85 × 10 ⁻³	3.21 × 10 ⁻⁶
	Control	2.10 × 10 ⁸	—	1.00 × 10 ⁻⁴
C	50 J m ⁻²	3.67 × 10 ⁷	1.09 × 10 ⁻³	n.d. ^d
	Control	8.80 × 10 ⁷	—	n.d. ^d
D	50 J m ⁻²	1.42 × 10 ⁸	3.11 × 10 ⁻³	2.14 × 10 ⁻⁵
	Control	2.39 × 10 ⁸	—	2.15 × 10 ⁻⁵

a. Recombination frequencies were determined as event per cell; the median of the positive recombinants (*lacS*⁺/*pyrEF*⁻) cfu ml⁻¹ with selection (5-FOA) were determined and divided by the median of the cfu ml⁻¹ from all observed colonies under non-selective conditions.

b. Mutation frequencies for strain P1 as event per cell.

c. No recombination events observed.

d. Not determined.

different probes (not shown). After UV irradiation and mixing of cells from two different species cellular aggregation was visualized with the probes (Fig. 2). Large, homologous cell clusters were formed as indicated by the homogeneously coloured green and red aggregates, respectively, as shown for *S. solfataricus* and *S. tokodaii* in Fig. 2A and for *S. acidocaldarius* and *S. tokodaii* in Fig. 2B. This indicated that cellular aggregation was based on a species-specific recognition process. Respective control experiments revealed a homogenous distribution of single cells and only few aggregates, demonstrating that the aggregate formation was indeed UV-induced and not a biased result of the FISH procedure (Fig. 2A and B, lower control panels). Only few, very large aggregates were occasionally observed that contained differentially stained cells. However, even in those large aggregates, no completely random mixing of colours was seen, but rather batches of cells of the same colour seemed to aggregate with batches of the other colour to form a huge aggregate. Quantitative analysis of the hybridized homogenous and heterogeneous aggregates confirmed that only a minor fraction of all formed aggregates consisted of two strains, i.e. *S. solfataricus* mixed with *S. tokodaii* (12.8 ± 6.9%) and *S. acidocaldarius* mixed with *S. tokodaii* (10.3 ± 4.5%). From the few aggregates seen in control experiments without UV irradiation a significantly higher proportion consisted of the two mixed strains with UV treatment (*S. solfataricus* × *S. tokodaii*: 40.6 ± 1.7%; *S. acidocaldarius* × *S. tokodaii*: 28.5 ± 2.5%). These data further demonstrate that the process of UV-induced aggregation is species-specific.

UV-inducible pili mediate increased exchange of chromosomal markers among *Sulfolobus* cells

The formation of aggregates in UV-treated cultures of *Sulfolobus* led to the hypothesis that these are used for

DNA exchange assisting DNA damage repair by homologous recombination. In order to test this hypothesis, mating experiments with a *lacS*/*pyrEF* double mutant of *S. solfataricus* (Table 3) defective in β-galactosidase and *de novo* uracil synthesis, respectively (Martusewitsch *et al.*, 2000), was mixed with wild-type cells of strain P1. The mating assay was based on the transfer of the β-galactosidase encoding reporter gene *lacS* from the wild-type strain P1 to the *lacS*-deficient strain PH1-M16. Recombinants (ex-conjugants) were screened for, which were 5-FOA-resistant (thus *pyrEF*⁻) and formed blue colonies when incubated with X-gal, thus *lacS*⁺. The recombination frequencies were up to three orders of magnitude higher than spontaneous mutations of the wild-type strain alone that resulted in the same phenotype (Table 1). No reversion events of the mutated *pyrEF* or *lacS* genes of the PH1-M16 (*lacS*⁻/*pyrEF*⁻) strain were observed with or without UV irradiation. The *lacS*⁺/*pyrEF*⁻ genotype of recombinants was verified by Southern Blot analyses (Fig. 3). No recombination events were observed without UV irradiation.

To better characterize the role of pili in DNA transfer the genes for the central ATPase UpsE and an integral transmembrane protein UpsF from the *ups* operon were removed via a marker-less exchange method in *S. acidocaldarius* (Wagner *et al.*, 2009). In this species the genetic toolbox is best developed and the knockout of *upsEF* genes is one of the first mutants that has been prepared via a single cross-over (Wagner *et al.*, 2009). Southern blotting confirmed the deletion in the mutant (Fig. 4). As expected we could not observe any pili in the deletion strain before and after UV irradiation, and no significant increase of aggregate formation (Fig. S2), confirming earlier results with *S. solfataricus* that the pili are necessary for aggregate formation (Fröls *et al.*, 2007).

We then used a series of *ups*/*pyrE* double mutants in mating experiments (Table 3). The assays detected trans-

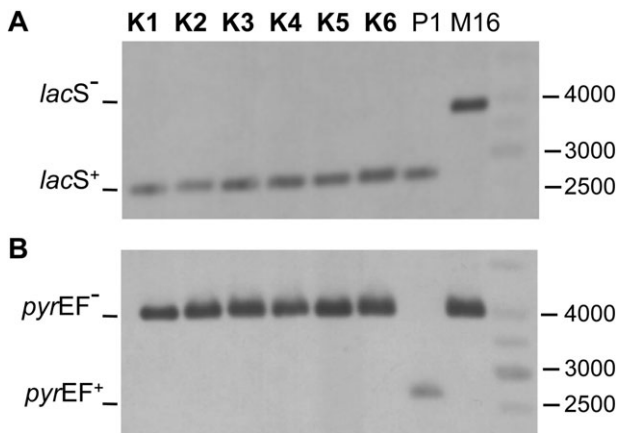


Fig. 3. Southern-blot analysis confirming the resulting (*lacS*⁻/*pyrEF*⁻) genotype of isolated positive recombinants (K1–K6), after mating of *S. solfataricus* P1 (*lacS*⁻/*pyrEF*⁻) with *S. solfataricus* M16 (*lacS*⁻/*pyrEF*⁻) using *lacS*-specific DNA probes (A) and *pyrEF*-specific DNA probes (B).

fer of chromosomal DNA from either parental strain to the other, leading to recombination events that restored the function of the biosynthetic gene (*pyrE*) inactivated by a different mutation in the two strains (Hansen *et al.*, 2005). To provide multiple options for this assay, two corresponding pairs of *pyrE* strains were constructed. Each pair included one mutant with a short deletion centred around *pyrE* nt 163, and one with a point mutation at *pyrE* nt 335 (see *Experimental procedures* and Table S2). The two pairs of strains differed with respect to their *upsEF* genotype; one pair (MR31 and JDS28) was *ups*⁺ (i.e. Pil⁺) the other pair (SA1 and DG253) was Δ *upsEF* (i.e. Pil⁻). Despite the fact that the *pyrE* marker spacing was the same in both crosses, MR31 mated with JDS28 yielded 20–30 Pyr⁺ recombinants, whereas SA1 with DG253 yielded none (Fig. 5). This indicated that the type IV pili made by the Ups system are necessary for DNA transfer even without UV treatment in *S. acidocaldarius*. We also evaluated 'unequal' mating in which one partner was Pil⁺ and the other Pil⁻. These matings yielded recombinants

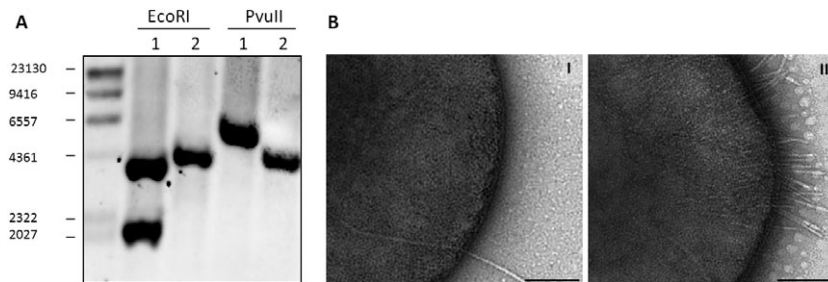


Fig. 4. Southern blot analysis of wild-type Pil⁺ *S. acidocaldarius* MR31 (1) and Pil⁻ (2) strain (A) to confirm knockout of *upsEF* genes. Expected fragment sizes for WT MR31: 3906 bp and 2128 bp for EcoRI and 6785 bp for PvuII (1). Expected fragment sizes for knockout of *upsEF*: 4593 bp for EcoRI and 5344 for PvuII (2). Electron micrographs of Pil⁻ strain with UV (BI) and Pil⁺ strain with UV (BII). Only flagella like structures can be observed on the cell surface of UV-treated Pil⁻ cells but no short straight UV pili (B). Scale bar is 0.2 μ m in length.

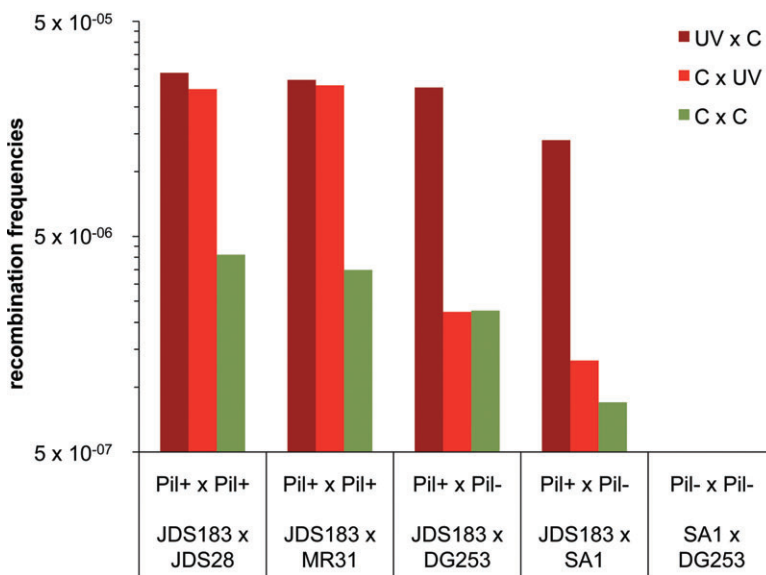


Fig. 5. Recombination frequencies of mating experiments with *S. acidocaldarius* wild-type and delta-pili mutants. Two different strains treated with (UV) or without (C) UV, were mixed together in different combinations and plated on selective media. All strains contained mutations in the *pyrE* gene (involved in *de novo* uracil biosynthesis) located at different positions, such that recombination between two strains can restore the wild-type phenotype. Bars represent the median of three or four independent mating experiments each (only one experiment in the case of delta pili \times delta pili mating). Recombination frequencies were calculated from the cfu obtained after direct plating upon UV treatment.

Table 2. Genotype analysis^a of recombinants of *S. acidocaldarius* from matings with and without prior UV treatment.

Mixed mating strains		No. of tested recombinants	<i>upsE</i> genotype of recombinants ^a		
			<i>pil</i> ⁻ (<i>upsE</i> ⁻)	<i>pil</i> ⁺ (<i>upsE</i> ⁺)	% <i>pil</i> ⁺ (<i>upsE</i> ⁺)
WT <i>pili</i> × Δ <i>pili</i> (JDS183 × SA1)	UVxC	16	3	13	81.25
	CxUV	16	4	12	75
	CxC	16	4	12	75
WT <i>pili</i> × Δ <i>pili</i> (JDS183 × DG253)	UVxC	15	5	10	66.7
	CxUV	16	6	10	62.5
	CxC	16	3	13	81.25

a. Analysis performed by PCR with primers flanking the *upsE* locus.

(Fig. 5), indicating that successful DNA exchange requires only one of the two parental strains to have *pili*.

We then evaluated 'unequal' (i.e. *Pil*⁺ and *Pil*⁻) combinations in which only one of the two parental strains was irradiated before mating. To provide multiple comparisons, members of isogenic *Pil*⁺ and *Pil*⁻ pairs (JDS28/DG253 or MR31/SA1) were each mated to a common *Pil*⁺ *pyrE* mutant, JDS183. As shown in Fig. 5, when one of the strains was UV-treated, an increase in yield of recombinants was only observed when the UV-irradiated strain was *Pil*⁺. UV treatment of only the *Pil*⁻ strain never elevated the recombination frequency (Fig. 5, Table S3). Finally, for assays in which a *Pil*⁺ and a *Pil*⁻ strain had been mated, the *upsE* genotype of recombinants was scored by PCR analyses (Table 2). Between 62% and 81% of the *pyrE*⁺ colonies were *upsE*⁺, meaning that at least two-thirds of the *Pyr*⁺ recombinants were *pili* producers.

Attempts to demonstrate marker recombination with lysed cells from one partner and UV-irradiated intact cells of a second partner failed in all cases, indicating that the DNA transfer does not involve the uptake of free DNA, as was also shown before (Grogan, 1996).

Increased fitness of *Pil*⁻ strains upon UV treatment

In order to analyse the survival of *Pil*⁺ and *Pil*⁻ strains upon UV treatment, the four different strains of *S. acidocaldarius* were exposed to different doses of UV light and subsequently plated (Fig. 6). A significant reduction in plating efficiency was observed for the *pili* knockout strains compared with *Pil*⁺ strains, indicating that the *pilus*-mediated process considerably increases the fitness of cells. The effect was particularly obvious at relatively low doses of UV light, i.e. 35 and 50 J m⁻², where approximately 60% and 40% of the wild-type cells survived, but

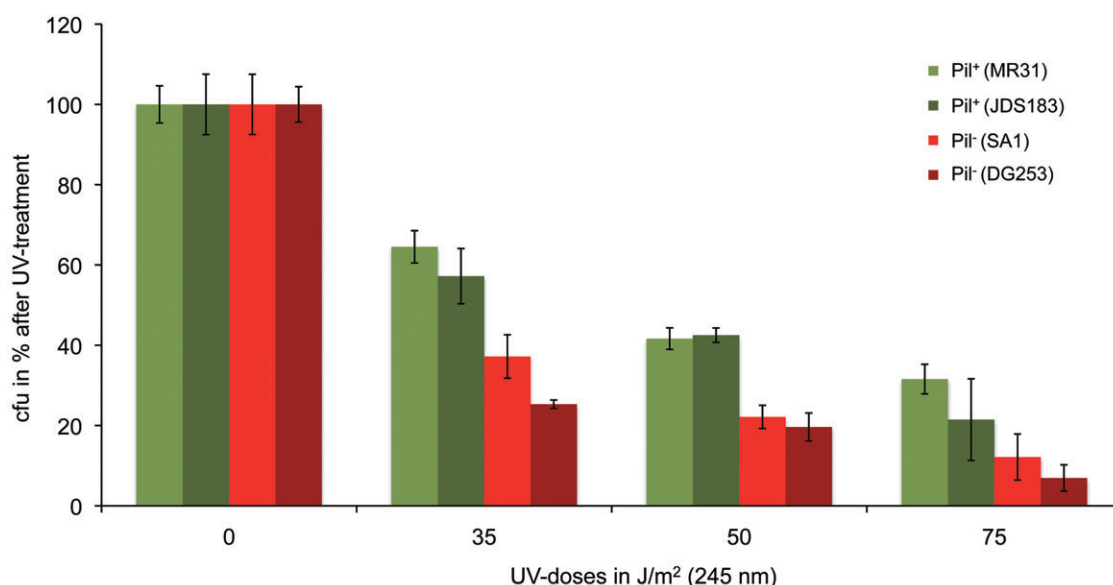


Fig. 6. Plating efficiency of *S. acidocaldarius* wild-type (green) and delta *pili* strains deficient in *pili* formation (red) treated with different UV doses. Results are based on three independent experiments. This figure is available in colour online at wileyonlinelibrary.com.

only around 30 and 20%, respectively, of the mutant strains (Fig. 6). The significance of these results was confirmed by statistic evaluation of the yielded survival rates (see Table S4). First a two-way ANOVA was performed to revise variance among the strains and the used UV doses. The calculation revealed highly significant differences (P -value < 0.001) among the tested strains (P -value 9.1×10^{-7}) and the UV doses (P -value 2.2×10^{-16}). This statistical evaluation confirmed that the cellular resistance to UV damage is dependent on the existence of a functional *ups* operon and mediated processes.

Discussion

Homologous recombination (HR) is a mechanism that is used in all domains of life for repair of double-strand DNA breaks (Dudas and Chovanec, 2004) and restart stalled DNA replication forks. Furthermore, HR is considered an important mechanism to maintain genomic cohesion among individuals of a bacterial or archaeal species (Papke *et al.*, 2007), but is also the prerequisite for exchange of accessory genomic functions, e.g. antibiotic resistance and pathogenicity islands. While it is generally accepted that genetic exchange among microbes must occur at high frequencies, relatively little is known about the conditions which trigger effective gene exchange among individuals of a microbial community. As in bacteria, a number of mobile genetic elements, such as viruses, plasmids and insertion sequences have been described in archaea, all of which can promote directly or indirectly HR. Conjugative plasmids have so far only been described in the crenarchaeal order *Sulfolobales* (Schleper *et al.*, 1995; She *et al.*, 1998; Stedman *et al.*, 2000). They seem to spread effectively among strains (Schleper *et al.*, 1995). However, the mechanism of conjugation is not understood and only few genes on these plasmids show remote similarity to genes involved in conjugation of bacteria (She *et al.*, 1998). In the archaeon *Haloferax volcanii* an effective exchange of chromosomal markers has been demonstrated (Rosenshine *et al.*, 1989) that seems to involve a mutual exchange of genomic material via cytoplasmic bridges. Similarly, frequent exchange of chromosomal markers, independent of the presence of known conjugative elements in the cells, has been demonstrated in plating experiments with *S. acidocaldarius* (Grogan, 2009) which increases with UV treatment (Schmidt *et al.*, 1999). Furthermore, molecular studies by Whitaker *et al.* (2005) on 60 different strains of *Sulfolobus islandicus* demonstrated homologous recombination for all six loci investigated. Frequent recombination among naturally occurring *Ferroplasma acidarmanus* strains resulting in a mosaic gene pool were described in a metagenomic study (Eppley *et al.*, 2007). Furthermore, Papke *et al.* (2004)

reported on vigorous recombination among *Halorubrum* strains with an identical 16S rRNA gene in a solar saltern that resulted in a population approaching linkage equilibrium. While frequent exchange of chromosomal markers has been described the mechanisms and driving forces underlying lateral gene transfer (LGT) remain largely unknown in archaea, and also in bacteria. The UV-inducible chromosomal DNA exchange in *Sulfolobus* that we describe here might not only represent an effective means to repair DNA by homologous recombination, it might also promote frequent DNA exchange in natural communities. We have observed up to three orders of magnitude higher recombination frequencies after UV treatment.

Different from many bacterial conjugation or DNA exchange processes described so far, the DNA transfer in *Sulfolobus* (i) is strongly inducible by UV treatment, (ii) involves the transfer of chromosomal markers and (iii) is dependent on the formation of type IV pili by at least one partner. UV treatment of only one (pili-producing) partner was sufficient to increase the yield of DNA exchange. This increase only occurred when the *Ups*⁺ partner was irradiated, whereas UV treatment of the partner without pili did not stimulate recombination. Furthermore, more than two-thirds of the *S. acidocaldarius* *Pyr*⁺ recombinants that resulted from mating between an *ups*⁺ and Δ *upsEF* strain were *Pil*⁺. We thus conclude that the high level of transfer seen after UV irradiation involves an active recruitment of DNA by a UV-damaged cell that produces pili. The DNA donor can be either a non-damaged or a UV-damaged cell (see Fig. 5). It has not been established, however, why a significant minority of recombinants from these matings are Δ *upsEF*. Possibilities include non-selected transfer of the *upsEF* deletion to the (originally *Ups*⁺) recipient, or reverse transfer of DNA between paired cells that brings *pyrE* DNA into the Δ *upsE* partner. It might, however, also be possible that the transfer is to some extent bidirectional with one partner being UV-activated and that our ex-recombinants are in part the results of mutual transfer of the two markers.

Interestingly, type IV pili of bacteria are usually not associated with conjugation or with cell-to-cell transfer of DNA but are rather involved in DNA uptake from the environment (Chen *et al.*, 2005) or in other processes, such as motility (Ayers *et al.*, 2010). However, in two cases type IV pili have recently been identified to be involved in cellular DNA transfer in bacteria (Kim and Komano, 1997; Carter *et al.*, 2010). Whereas the conjugative type IV pilus of R64 is plasmid encoded (Kim and Komano, 1997), a type IV pilus involved in conjugation is encoded in the pathogenicity island PAPI-1 in *Pseudomonas* strains. Transmission of the island is dependent on a factor from the core genome, which probably restricts the range of bacteria that can participate in the exchange of

this genetic island (Carter *et al.*, 2010). Compared with this, the *Sulfolobus* systems seems to be fully chromosomally derived: the Ups type IV pili are genome encoded, no GC skew is evident to the surrounding genomic sequences and their genetic context does not indicate that they are derived from a former conjugative plasmid. Furthermore, they do not only transfer a particular chromosomal island or plasmid, but rather transfer unspecific regions or perhaps even the whole genome during UV-induced DNA repair (Wood *et al.*, 1997; and *S. solfataricus* assay in this study).

A variety of type IV pili have been described in archaea and their prepilins are processed by a dedicated type IV prepilin signal peptidase PibD/FlaK like the bacterial ones. The pili have been shown to be involved in surface motility, swimming, surface attachment and biofilm formation (Albers and Pohlschroder, 2009; Koerdt *et al.*, 2010). As the structural subunits of the ups system in the different *Sulfolobales* strains are highly conserved, but the appearance of the pili strikingly different, we propose that the species specificity of aggregation depends on the recognition of the pilin subunits with the cell surface. Successful cell aggregation and subsequent DNA exchange will only take place, when the pili have determined the 'host' cell being from the same species ensuring that DNA exchange is restricted to species from within the same gene pool. However, it could as well be possible that additional species-specific surface markers function in the recognition process.

In conclusion we describe a novel UV-inducible DNA transfer in hyperthermophilic *Sulfolobales*. It is mediated by type IV pili and loss of these pili reduces fitness of cells after UV damage, either by abolishing the DNA transfer process or by another as yet unknown mechanism. The DNA transfer triggers homologous recombination among cells in a population and might thus significantly promote horizontal gene transfer in naturally occurring communities. It remains to be shown if UV-induced DNA exchange mechanisms are found more widespread in other archaea and bacteria.

Experimental procedures

Growth conditions and UV light exposure

All *Sulfolobus* strains were grown at 78°C in Brock's medium (Brock *et al.*, 1972) or alternatively in a less complex mineral mixture (Grogan and Gunsalus, 1993), adjusted to pH 3 with sulphuric acid and supplemented with 0.1% (w/v) of trypton or NZ-Amine; 0.2% (w/v) xylose and 10 µg ml⁻¹ uracil when necessary. Growth of cells was monitored by optical density measurements at 600 nm. For solid media the medium was supplemented with 1.5% gelrite. Plates were incubated for 5–6 days at 78°C. UV light exposure of *S. solfataricus* and *S. tokodaii* was performed for a few seconds according to the desired UV dose as described in Fröls *et al.* (2008). For UV

light exposure of *S. acidocaldarius* 25 ml of culture (OD₆₀₀ 0.2–0.4) was treated with a defined UV dose (254 nm, Spectroline, UV cross-linker) in a plastic Petri dish and cells were immediately plated. Samples taken at different time points were analysed in phase-contrast microscopy, live and dead assay and electron microscopy. Optimal pili formation was found 3 h after UV treatment for *S. acidocaldarius* and *S. solfataricus* and 6 h for *S. tokodaii*.

Electron microscopy analysis

For image processing, cells were negatively stained with 2% uranyl acetate on carbon-coated copper grids. Transmission electron microscopy was performed on a Philips CM10 electron microscopy operating at 100 kV.

Light microscopy and quantitative analysis of aggregates

Preparation of cells and light microscopic analysis was performed as described by Fröls *et al.* (2008). To quantify the number of aggregates, the cells were counted until at least 1000 single cells were observed. For the quantitative analysis the percentage of cells in aggregates (≥ 3 cells) against total amount of cells was calculated.

Fluorescence in situ hybridization

For FISH samples of single and mixed *Sulfolobus* strains, with and without UV treatment, were used. For strain combination experiments, cultures were mixed in a stoichiometric ratio of 1:1, directly upon treatment. Flasks were stored in the dark for 15 min at room temperature and incubation was continued at 78°C for 6 h with mild shaking at 150 r.p.m. After 6 h of incubation, samples were diluted 1:10 in Brock basal salt medium, 10 µl of culture was placed into a well on a FISH microscope slide (Marienfeld) and dried at RT. For fixation 10 µl of 37% formaldehyde was placed on top of each well and incubated for 20 min at RT. Afterwards formaldehyde was replaced by 10 µl of 1× PBS buffer and this was incubated for 2–3 min at RT to wash the cells. Fixed samples on the microscope slides were directly used for hybridization or stored at –20°C. FISH was carried out as described in detail elsewhere (Daims *et al.*, 2005). For probe sequences please see Table S1.

Following FISH, slides were embedded in Citifluor (Citifluor AF1; Citifluor, London UK) and examined using a confocal laser scanning microscope (LSM 510Meta; Carl Zeiss, Jena, Germany). For the quantification of the aggregates three technical replicates were used and hybridized in replicates respectively (resulting in a total of six hybridizations per mixing experiment). After FISH, hybridizations were recorded using the tile scan function of the LSM510 Meta, allowing basically the recording of the whole microscopic well. Subsequently, aggregates were counted manually following the above-described criteria, with ≥ 3 cells counted as an aggregate. Furthermore, the aggregates were classified as species-specific (= aggregate with cells from only one species) or as mixed aggregate. In the latter case, an aggre-

Table 3. Strains used in this study.

Strain	Genotype or description	Source or reference
<i>S. acidocaldarius</i> MR31	Deletion of 18 bp in <i>pyrE</i> (nt 154–171)	Reilly and Grogan (2001)
<i>S. acidocaldarius</i> JDS28	Point mutation in <i>pyrE</i> (A > T at nt 335)	Sakofsky <i>et al.</i> (2011)
<i>S. acidocaldarius</i> JDS183	Frameshift mutation in <i>pyrE</i> (duplicated T at nt 44).	Grogan and Hansen (2003)
<i>S. acidocaldarius</i> SA1	Δ <i>upsEF</i> in MR31	D. W. Grogan
<i>S. acidocaldarius</i> DG253	Δ <i>upsEF</i> , point mutation in <i>pyrE</i> (A > G at nt 335)	D. W. Grogan
<i>S. solfataricus</i> P1 (DSM1616)	Wild type	DSMZ
<i>S. solfataricus</i> PBL2025	<i>S. solfataricus</i> 98/2 Δ (<i>ss03004</i> – <i>3050</i>)	Schelert <i>et al.</i> (2004)
<i>S. solfataricus</i> Δ <i>upsE</i>	<i>S. solfataricus</i> PBL2025 Δ <i>ss0120</i>	Fröls <i>et al.</i> (2008)
<i>S. solfataricus</i> PH1-M16	<i>S. solfataricus</i> P1 Δ <i>lacS</i> , Δ <i>pyrEF</i>	Martusewitsch <i>et al.</i> (2000)
<i>S. tokodaii</i> (DSM16993)	Wild type	DSMZ

gate consisted of the two mixed strains, whereupon the mixing ratio was regarded as irrelevant; e.g. a mixture of five cells from one strain and a single cell of another strain was classified as mixed aggregate. In total more than 1000 aggregates were counted.

Plasmid cloning for gene deletion and mutant construction

The upstream and downstream flanking regions of *Saci_**upsEF* were amplified using primer pairs 1-forward/11-reverse (GCGGGCCGCGGGATAGAAAGTTGAAAGTGGAC/GCGCCCGGATCCAGACAGAAAAATGAAAGTTATCAAGG) and 12-forward/13-reverse (CGCGCCCGGGCAAGTCTTATATAGAATATACAGGAGACG/CGCGCTGCAGCATTTAGGAGAAGAGCGAC) respectively. PCR products were cloned using *SacII*/*Bam*HI for the upstream flanking region and *SmaI*/*PstI* digestion for the downstream region in Δ 2*pyrEF*, a vector containing the *pyrEF* cassette for selection (Wagner *et al.*, 2009), yielding pGA5. pGA5 was methylated as described before (Kurosawa and Grogan, 2005) and transformed into MR31, integrants were then selected on plates lacking uracil. Subsequently they were streaked out on second selection plates containing 5-FOA to force looping out of the plasmid by homologous recombination. Resulting colonies were tested by PCR for successful deletion of the *upsEF* locus using primer pairs 16-forward/15-reverse (GGCATGATCAGGTATTAGCAGAG/CCATAAGTATGCAGTTATCAGCTCG) and 1-forward/13-reverse (GCGGGCCGCGGGAATAGAAAGTTGAAAGTGGAC/CGCGCTGCAGCATTTAGGAGAAGAGCGAC). DNA sequencing on a PCR product 1000 bp larger than the flanking regions of the *upsEF* region (created with 44-forward/17-reverse: GGGTAGTATATGATGCTATAAGGTAAGT/CCGTAACAGTATATGTCTCTCCTGCAATC) confirmed the correctness of the mutants.

Southern hybridization

Genomic DNA (5 µg) was digested with *EcoRI* and *PvuII* and separated on a 0.8% agarose gel. Southern blotting was performed using standard procedures. DNA hybridization was performed in 50% formamid, 5× SSC, 2% blocking reagent, 0.1% Na-lauroylsarcosine, 0.02% SDS. PCR products of *pyrEF* (using GTCATCTCTGGTCAAGT-CAAGC GACGAACTACGTGTC and GAACTTGATATGAGAGAG

GTTTAT-CCATTGC), *lacS* (using GGCATGGATCAGGTATTAGCAGAG and CCATAAGT-ATGCAGTTATCAGCTCG) and *Saci_**upsEF* (using GCGGGCCGCGGGATATAG-AAGTTGAAAGTGGAC and CGCGCTGCAGCATTTAGGAGAAGA GCGAC). PCR products were digoxigenin-labelled with the HighPrime Kit (Roche, the Netherlands). Detection was performed with a Luminescent Image Analyser (FUJIFILM).

Recombination assays for *S. acidocaldarius*

DNA exchange between *S. acidocaldarius* cells was assayed by selecting prototrophic (*Pyr*⁺) recombinants of two *pyrE* mutant strains (see also Table 3). The *ups*⁺*pyrE* strains were MR31, JDS28 and JDS183 (Grogan *et al.*, 2001; Reilly and Grogan, 2002). Strain MR31 has an 18 bp deletion removing *pyrE* nt 154–171, JDS28 contains a transversion mutation (A to T) at nt 335 of the *pyrE* gene; and strain JDS183 contains a frameshift mutation (duplication of T) at nt 44. The Δ *upsEF* strain DG253 is essentially isogenic with strain JDS28; it was derived from the *pyr*⁺ Δ *upsEF* SA1 strain by 5-FOA selection (Kondo *et al.*, 1991), and was found by PCR and sequencing to contain a transition mutation (A to G) at *pyrE* nt 335 of *pyrE* gene. Liquid cultures were grown at 78°C and harvested at OD₆₀₀ = 0.15–0.35. Pellets were resuspended to a cell density of about 2 × 10⁸ cells ml⁻¹ (Grogan, 1996). UV irradiation was performed as described above. Surviving of cells was determined by serial 1:10 dilution and spread plating on uracil-supplemented xylose-tryptone plates. Phenotypic reversion of the *pyrE* mutations was assayed by plating 0.1 ml of each suspension on selective (unsupplemented xylose-tryptone) plates. Recombination was assayed by spreading a mixture representing 50 µl of each of two suspensions on selective unsupplemented xylose-tryptone plates. Plates were incubated for 5–6 days at 78°C.

Mating assay for *S. solfataricus*

For each mating experiment a minimum volume of 60 ml of an exponentially grown *S. solfataricus* culture (OD₆₀₀ 0.2–0.4) was used. After UV treatment *S. solfataricus* strains P1 (*lacS*⁺/*pyrEF*⁺) and PH1-M16 (*lacS*⁻/*pyrEF*⁻) were mixed with equal amounts of cells. Flasks were stored in the dark for 15 min at room temperature, uracil (12.5 µg ml⁻¹ final concentration) was added. Incubation was then continued without keeping the flasks in the dark at 78°C for 6 h with mild

shaking at 100 r.p.m. Samples were plated after dilution in Brock's basal salt pH 3, without carbon sources. Beta galactosidase activity was assayed by spraying the colonies (which appeared after 5–7 days) with X-Gal. To determine the number of colony-forming units (cfu) without selection, cells were plated on Brock's basal salt solid media with D-arabinose (0.2%) tryptone (0.1%) and 10 µg ml⁻¹ uracil as additives.

To analyse the reversion and mutation frequencies, the median of the cfu ml⁻¹ with selection (5-FOA) was determined and divided by the median of the cfu ml⁻¹ under non-selective conditions. To determine the recombination frequency, the median of the positive recombinants (*lacS*⁺/*pyrEF*⁻) cfu ml⁻¹ with selection (5-FOA) was determined and divided by the median of the cfu ml⁻¹ from all observed colonies under non-selective conditions. To identify positive mates (*lacS*⁺/*pyrEF*⁻) cfu ml⁻¹ with selection (5-FOA), more than 13.000 cells were counted and 788 positive colonies were identified. In the case of the control experiments in total more than 11 000 cells were counted and five events were identified, which correspond to the determined mutation frequency of *S. solfataricus* strain P1 of 10⁻⁵ events per cell.

Acknowledgements

We thank Michael Wagner (Department of Microbial Ecology, University of Vienna) for generous access to his confocal laser scanning microscope, and Jens Riede for help with the statistical analysis. M.A. was supported by an ALW grant from the Dutch Science Organization (NWO). S.-V.A. was supported by a VIDI grant from NWO and intramural funds of the Max Planck Society. D.W.G. was supported by National Science Foundation grant MCB0543910. D.T. and M.v.W. received funds from the European network SOLAR MRTN-CT-2006-033499. K.S. was supported by Austrian Science Fund FWF Grant P20775.

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